IN THE SPECIFICATION

Approval by the Examiner of Figures 4-17 is requested.

Page 4, replace the third paragraph starting at line 17 with the following:

This invention provides for the active site mapping of enzymes which catalyse covalent modification including, but not limited to phosphorylation, acylation, dephosphorylation in which a fixed residue (hereafter known as the catalytic residue) such as a tyrosine, serine, threonine, histidine, aspartic acid residue or any other residue containing an appropriate side chain is modified. The method of the invention has an additional level of complexity over and above that of the self-deconvoluting libraries described in W097/42216 and Example 5 (the content of which is incorporated herein by reference, where legally permissible).

Page 8, insert the following on line 20.

Figures 4 to 17. Component distributions in the plates of a library matrix.

Page 10, replace the third paragraph starting at line 15 with the following:

Library peptides were made with biotin tags, which allowed peptide capture on strepavidin-coated microtitre plates. Detection of phosphotyrosine was achieved using anti-phosphotyrosine antibody detection in an ELISA assay using tetramethylbenzidine substrate and recording absorbance at 450 nm. Background absorbance readings of 0.1 to 0.2 were recorded while the highest substrate peptide value was 1.5. Deconvolution of the hit peptides was performed as described in WO 97/42216 and Example 5. Clear defined substrates were deconvoluted in library sub-sets 1 to 4, but not in 5. This probably reflects the absolute requirement of ZAP-70 for an amino acid residue in the -1 position.

Page 12, replace the second paragraph starting at line 4 with the following:

In the second application of this invention, a recombinant form of the human Syk enzyme was used in an *in vitro* phosphorylation reaction to phosphorvlate the five substrate sub-libraries which scan the sequence -4 to +4 around a central tvrosine residue, as previously performed for the ZAP-70 library. Detection of phosphotyrosine was achieved using anti-phosphotyrosine antibody detection in an ELISA assay using tetramethylbenzidine substrate and recording absorbance at 450 nm. Background absorbance readings of 0.10 were recorded while the highest substrate peptide value was 1.46. Deconvolution of the hit peptides was performed as described in WO 97/42216 and Example 5. Clear defined substrates were deconvoluted in all library sub-sets.

Page 12, replace the third paragraph starting at line 14 with the following:
In the third application of this invention, a recombinant form of the human CSK enzyme was used in an *in vitro* phosphorylation reaction to phosphoiylate the five substrate sub-libraries which scan the sequence -4 to +4 around a central tyrosine residue, as previously performed for the ZAP-70 library. Detection of phosphotyrosine was achieved using anti-phosphotyrosine antibody detection in an ELISA assay using tetramethylbenzidine substrate and recording absorbance at 450 nm. Background absorbance readings of 0.04 were recorded while the highest substrate peptide value was 0.22. Deconvolution of the hit peptides was performed as described in WO 97/422 16 and Example 5. Clear defined substrates were deconvoluted in all library sub-sets.

Pages 12-13, replace the fourth paragraph starting at line 24 with the following:
In the fourth application of this invention, a recombinant form of the Abelson murine leukaemia virus protein tyrosine kinase v-Abl was used in an *in vitro* phosphorylation reaction to phosphorylate the library sub-set 4 which scans the sequence -1 to +3 around a zero position tyrosine residue, as previously performed for the ZAP-70 library. Detection of phosphotyrosine was achieved using anti-phosphotyrosine antibody detection in an ELISA assay using tetraniethylbeuzidine substrate and recording

absorbance at 450 nm. Background absorbance readings of 0.11 were recorded while the highest substrate peptide value was 0.32. Deconvolution of the hit peptides was performed as described in WO 97/42216 and Example 5. Clear defined substrates were deconvoluted in the library sub-set.

Page 13, replace the second paragraph starting at line 7 with the following:
In the fifth application of this invention, the invention was used to map the substrate specificity of a protein serine or serine/threonine kinase (which include I-kappa B kinase beta and cAMP-dependent protein kinase [cAPK]). A protein serine or serine/threonine kinase enzyme was used in an *in vitro* phosphorylation reaction to phosphorylate the five substrate sub-libraries which scan the sequence -4 to +4 around a central serine residue. The library was synthesised as the protein tvrosine kinase ZAP-70 library save that the tyrosine fixed residues were replaced with a seine which was then scanned through the five sub-libraries. Detection of phosphoserine was achieved using anti-phosphoserine antibody detection in an ELISA assay using tetramethylbenzidine substrate and recording absorbance at 450 nm. Deconvolution of the hit peptides was performed as described in WO 97/422 16 and Example 5.

Page 20, replace the third paragraph starting at line 22 with the following:
The best substrates were identified as those which gave the highest amount of phosphate incorporation. The library subsets were deconvoluted according to the teaching of W097/42216 and Example 5: this gives an immediate determination of the unique sequence of any phosphorylated motif without the need for further synthesis or sequencing. (Figure 2 [SEQ ID Nos. 7,8,9,10]).

Page 21, replace the third paragraph starting at line 21 with the following:
The mapping and assessment of the catalytic site was performed as detailed in Example 1. The substrate preferences were deconvoluted as detailed in WO 97/42216 and Example 5 and are detailed below.

Page 22, insert the following starting on line 25:

Example 5

Deconvolution methodology from WO97/42216.

Libraries or sub-libraries are arranged as two orthogonal sets of mixtures of compounds in solution providing two complementary combinatorial libraries indexed in two dimensions for autodeconvolution. These are referred to as primary and secondary libraries.

The general concept of two orthogonal sets of mixtures indexed in two dimensions can be applied to various permutations of numbers of wells, plate layout, number of permutations per mixture etc. However, according to the invention the numerical interrelationship is defined as indicated below for libraries containing compounds with four variable groups B, C, D and E.

General Deconvolution Formulae

-Bb-Cc-Dd-n(Ee)- (I)

1) Primary and Secondary plates preferably have the same number of compounds per well [X]: otherwise there are two values, having X_p and X_s respectively.

2) The primary library comprises [np] plates.

If Rp.Cp=Rs.Cs, then the number of plates in the secondary library is also [np]. If not, the number of plates in the secondary library [ns] is:

$$ns = \frac{Rp \times Cp \times np}{Rs \times Cs}$$

e.g., a primary library of np = 4, Rp = 8, Cp = 10 can be set out in an Rs = 4, Cs = 5 secondary library with the number of plates equal to:

$$ns = \frac{8 \times 10 \times np}{4 \times 5}$$

= 16 plates.

Number of compounds per well

-Bb-Cc-Dd-np(Ee)- (1)

Number of possible combinations [k] is given by:

$k = b \times c \times d \times np \times e$ (2)

When number of wells on a plate = [N], number of compounds per well = [X] and number of plates = [np].

$k = X \times N \times np$ (3)

However, number of wells [NI is also defined by the number of rows [Rp] and number of columns [Cp].

$N = Rp \times Cp$ (4)

Combining (3) and (4).

$k = X \times Rp \times Cp \times np$ (5)

Combining (2) and (5)

$b \times c \times d \times np \times e = X \times Rp \times Cp \times np$ (6)

Cancelling [np] from both sides of the equation:

$b \times c \times d \times e = X \times Rp \times Cp$ (7)

Two of the variables (e.g., b and c) on the left side of the equation must each be equal in number to the number of columns [Cp], whilst a remaining variable (e.g., d) on the left side must be equal in number to the number of rows [Rp]. So:

$[Cp]^2 \times Rp \times e = X \times Rp \times Cp$ (8)

Cancelling [Cp] and [Rp] from both sides of the equation.

$Cp \times e = X$ (9)

where [e] is the number of variants along a fixed row; and if Rp = Cp, then $Rp \times e = X$.

Example for a 10 x 10 x 8 x 8 format over 4 plates:

$np \times e = 8 \Rightarrow e = 2$

$10 \times 2 = X$

$X = 20$.

From an understanding of the general deconvolution formulae shown above, those skilled in the art will readily appreciate that the advantageous results of self-deconvolution according to WO97/42216 are obtainable utilising a number of different arrangements of wells, plate layouts, mixtures etc.

The technique will be illustrated by reference to a model system for screening a protease with a two complementary compound libraries, LI and L2, each contain n x 1600 compounds, of the type $A\text{-}B_{1\text{-}10} – C_{1\text{-}10}\text{-},,\text{-}D_{1\text{-}8}\text{-},\text{-}n(E_{1\text{-}2})$ -F-G [II], in which.

A = a fluorescor internally quenched by F, preferably an unsubstituted or substituted anthranilic acid derivative, connected by-an amide bond to B B, C, D, E, are natural or unnatural amino acid residues connected together by suitable bonds, although B, C, D and E can be any set of groups.

F = a quencher capable of internally quenching the fluorescor A, preferably an unsubstituted or substituted 3-nitrotyrosine derivative.

G = optionally present and is a hydrophilic moiety, preferably an aspartyl amide moiety. If present, G advantageously ensures that all compounds in the library are imparted with aqueous solubility. Also, G should not be a substrate for any type of enzyme.
n = any integer between 1 and 4 inclusive.

The numbers represented in subscript following residues B, C, D and E refer to the number of possibilities from which those residues are selected. Thus, by way of illustrative example, A-B_{1-5} -C-D-E_{1-2}-F-G represents a mixture of the following ten compounds.

A-B_1-C-D-E_1-F-G
A-B_2-C-D-E_1-F-G
A-B_3-C-D-E_1-F-G
A-B_4-C-D-E_1-F-G
A B_5-C-D-E_1-F-G
A-B_1-C-D-E_2-F-G
A-B_2-C-D-E_2-F-G
A-B_3-C-D-E_2-F-G
A-B_4-C-D-E_2-F-G
A B_5-C-D-E_2-F-G

The general combinatorial formula for each library can be expressed as:

A_1-B_{10}-C_{10}-D_8-n(E_2) –F_1-G_1 (III)

providing 1 x 10 x 10 x 8 x n x 2 x 1x 1 = 1600n is compounds.

Both compound libraries, L1 and L2, of the above type are synthesized using solid phase techniques using the Multipin approach" such that each library contains 1600n compounds as 80n mixtures of 20 distinct, identifiable compounds. These 20 component mixtures are then placed separately into each of 80 wells of a 96 well plate (the other two lanes are used for control experiments) and then screened against a known quantity of the protease.

Thus it is an important that regardless of the number of compounds contained in the two libraries L1 and L2 (e.g., in the preferred embodiment 1600n, where n = any integer between 1 and 4) the libraries themselves are complementary and amenable to deconvolution without recourse to resynthesis.

The general description of the library layout will now be described with reference to Figures 4 to 17 which exemplify component distributions in the plates of a library matrix;

For example, when n = 1 and the library contains 1600 compounds, in the first column of the first row (Al) (Fig. 4) in the first plate (P1) of the library L1, (hereinafter designated as location A1, P1, L1) there will be one C component (C_1), one D component (D_1), the ten B components, and the two E components (E_l and E_2) (Fig. 5). In the tenth column of the first row (A10) in the first plate (P1) of the library L1 (hereinafter designated as location A10, P1, L1), there will be one C component (C_{10}), one D component (D_1), the ten B components, and the two E components (E_1 and E_2). In the tenth column of the eighth row (H_{10}) in the first plate (P1) of the library L1 (hereinafter designated as location H10, P1, L1), there will be one C component (C_{10}) one D component (D_8), the ten B components, and the two E components (E_1 and E,). Hence all 1600 components are present in the one plate, because the 80 wells each contain 20 components.

A second complementary library is synthesised as follows (Fig. 6). In the first column of the first row (A1) of the first plate (P1) of the library L2 (hereinafter designated as location A1, P1, L2), there will be ten C components, two D components (D_3 and D_4), one B component (B_1), and one E component (E_1). In the tenth column of the first row (A10) of the first plate (P1) of the library, L2 (hereinafter designated as location A10, P1, L2), there will be ten C components, two D components (D_1 and D_2), one B component (B_{10}), and one E component (E_1). In the first column of the second row (B1) of the first plate (P1) of the library L2 (hereinafter designated as location B1, P1, L2), there will be ten C components, two D components (D_1 and D_2), one B component, B_1 and one E component, E_2. In the tenth column of the second row (B10) of the first plate (P1) of the library, L2 (B10,P1,P2) there will be ten C components, two D components (D_1 and D_2), one B component, B_1, and one E component, E_2. Hence only the first two rows are used to accommodate 400 compounds in total.

In the first column of the first row (A1) of the second plate (P2) of the library L2 (hereinafter designated as location A1, P2, L2), there will be ten C components, two D components (D_3 and D_4), one B component (B_1), and one E component (E_1) (Fig. 7). In the tenth column of the first row (A10) of the second plate (P2) of the library, L2, (hereinafter designated as location A10, P2, L2), there will be ten C components, two D components (D_3 and D_4), one B component (B_{10}), and one E component (E_1). In the first column of the second row (B1) of the second plate (P2) of the library L2 (hereinafter designated as location B1, P2, L2), there will be ten C components, two D components (D_3 and D_4), one B component (B_1), and one E component (E_2). In the tenth column of the second row (B10) of the second plate (P2) of the library L2 (B10, P2, L2), there will be ten C components, two D components (D_3 and D_4), one B component (B_{10}), and one E component (E_2). Hence only the first two rows are used to accommodate 400 compounds in total.

In the first column of the first row (A1) of the third plate (P3) of the library L2 (hereinafter designated as location A1, P3, L2), there will be ten C components, two D components (D_5 and D_6), one B component (B_1), and one E component (E_1) (Fig. 8). In the tenth column of the first row (A10) of the third plate (P3) of the library L2 (hereinafter designated as location A10, P3, L2), there will be ten C components, two D components (D_5 and D_6), one B component (B_{10}), and one E component (E_1). In the first column of the second row (B1) of the third plate (P3) of the library L2 (hereinafter designated as location B1, P3, L2). there will be ten C components, two D components (D_5 and D_6), one B component (B_1), and one E component (E_2). In the tenth column of the second row (B10) of the third plate (P3) of the library L2 (B10, P3, L2), there will be ten C components, two D components (D_5 and D_6), one B component (B_{10}), and one E component (E_2). Hence only the first two rows are used to accommodate 400 compounds in total.

In the first column of the first row (A1) of the fourth plate (P4) of the library L2 (hereinafter designated as location A1, P4, L2), there will be ten C components, two D components (D_7 and D_8), one B component (B_1), and one E component (E_1) (Fig. 9). In the tenth column of the first row A10) of the fourth plate (P4) of the library L2 (hereinafter designated as location A10, P4, L2), there will be ten C components, two D components (D_7 and D_8), one B component (B_{10}), and one E component (E_1). In the first column of the second row (B1) of the fourth plate (P4) of the library L2 (hereinafter designated as location is B1, P4, L2), there will be ten C components, two D components (D_7 and D_8), one B component (B_1), and one E component (E_2). In the tenth column of the second row (B10) of the fourth plate (P4) of the library L2 (B10, P4, L2) there will be ten C components, two D components (D_7 and D_8), one B component (B_{10}), and one E component (E_2). Hence only the first two rows are used to accommodate 400 compounds in total.

In this fashion two complementary libraries, L1 and L2 are prepared. In library L1, each of the 80 of wells contains a mixture of 20 components providing 1600 compounds for screening. In library L2, four plates are used in which only the first two rows are employed, providing 20 wells of 20 components per well per plate, and furnishing the same 1600 compounds as are present in library L1, but in a format in which no two compounds found together in library L1 will be found together in library L2.

Thus it is important that the compounds contained in the two libraries L1 and L2 are themselves complementary, in that any two compounds which are found together in a 20 component mixture in the same location (e.g., A1, P1, L1) in library L1, are not found together in any of the 20 component mixtures in any location of the library L2.

Thus, for example, with reference to the primary library P1 L1 of Figure 5 and the secondary libraries P1 L2, P2 L2, P3 L2 and P4 L2 of Figures 6-9 it is possible to deconvolute an exemplary sequence.

$$-B_2\ -C_3\ -D_4-\ E_1$$

If the library is a FRET library and this sequence is a substrate fluorescence will occur in P1 L1 at C_3D_4 This gives the information that the substrate is:

$$?\ -C_3-D_4-\ ?$$

If fluorescence occurs in P2 L2 at B_2E_1, it indicates a substrate:

$$-B_2-?-?-E_1-$$

The confirmation of the substrate as:

$$-B_2-C_3-D_4-E_1$$

should be provided by non-fluorescence of P1 L2, P3 L2 and P4 L2 which all contain -B_2-C_3-X-E_1- where X is not D.

In practice it is likely that more than one sequence will result in a substrate. Information as to which positions B-C-D-E- are sensitive to change (i.e., require a specific group) and which are insensitive (i.e., can tolerate more than one choice of group) in the context of the whole sequence gives valuable SAR data which can be used to model and/or synthesise related compounds.

In analogous examples, where separately n = 2, 3 or 4, extra plates are constructed in library L1 format to accommodate the component pairs E_3 and E_4 (n = 2), E_5 and E_6 (n = 3), and E_7 and E_8 (n = 4), respectively. For the respective deconvolution libraries of the type L2, the respective rows in the plates P1, P2, P3, and P4, are increasingly filled with the paired components D_1 and D_2, D_3 and D_4, and D_5 and D_6, and D_7 and D_8, respectively.

For example, when n = 3, and the library contains 4800 compounds, in the first column of the first row (A1) in the first plate (P1) of the library L1 (hereinafter designated as location A1, P1, L1) there will be one C component (C_1), one D component (D_1), the ten B components, and the two E components (E_1 and E_2). In the tenth column of the first row (A10) in the first plate (P1) of the library L1 (hereinafter designated as location A10, P1, L1) there will be one C component (C_{10}), one D component (D_1), the ten B components, and the two E components (E_1 and E_2). In the tenth column of the eighth row (H10) in the first plate (P1) of the library L1 (hereinafter designated as location H10, P1, L1) there will be one C component (C_{10}), one D component (D_8), the ten B components, and the two E components (E_1 and E_2). Hence 1600 components are present in the one plate, because the 80 wells each contain 20 components.

In the first column of the first row (A1) in the second plate (P2) of the library L1 (hereinafter designated as location A1, P2, L1) there will be one C component (C_1), one D component (D_1), the ten B components, and the two E components (E_3 and E_4). In the

tenth column of the first row (A10) in the second plate (P2) of the library L1 (hereinafter designated as location A10, P2, L1) there will be one C component (C_{10}), one D component (D_1), the ten B components, and the two E components (E_3 and E_4). In the tenth column of the eighth row (H10) in the second plate (P2) of the library L1 (hereinafter designated as location H10, P1, L1) there will be one C component (C_{10}), one D component (D_8), the ten B components, and the two E components (E_3 and E_4). Hence 1600 components are present in the one plate, because the 80 wells each contain 20 components.

In the first column of the first row (A1) in the third plate (P3) of the library L1 (hereinafter designated as location A1, P3, L1), there will be one C component (C_1), one D component (D_1), the ten B components, and the two E components (E_5 and E_6). In the tenth column of the first row (A10) in the third plate (P3) of the library L1 (hereinafter designated as location A10, P3, L1) there will be one C component (C_{10}), one D component (D_1), the ten B components, and the two E components (E_5 and E_6). In the tenth column of the eighth row (H10) in the third plate (P3) of the library L1 (hereinafter designated as location H10, P3, L1) there will be one C component (C_{10}), one C component (C_8), the ten B components, and the two E components (E_5 and E_6). Hence 1600 components are present in the one plate, because the 80 wells each contain 20 components. In total the three plates, P1, P2 and P3, contain 1600 compounds/plate 4800 compounds in total.

For example, when n = 4, and the library contains 6400 compounds, in the first column of the first row (A1) in the first plate (P1) of the library L1 (hereinafter designated as location A1, P1, L1) there will be one C component (C_1), one D component (D_1), the ten B components, and the two E components (E_1 and E_2) (Fig. 10). In the tenth column of the first row (A10) in the first plate (P1) of the library L1 (hereinafter designated as location A10, P1, L1) there will be one C component (C_{10}), one D component (D_1), the ten B components, and the two E components (E_1 and E_2). In the tenth column of the

eighth row (H10) in the first plate (P1) of the library L1 (hereinafter designated as location H10, P1, L1) there will be one C component (C_{10}), one D component (D_8), the ten B components, and the two E components (E_1 and E_2). Hence all 1600 components are present in the one plate, because the 80 wells each contain 20 components.

In the first column of the first row (A1) in the second plate (P2) of the library L1 (hereinafter designated as s location A1, P2, L1) there will be one C component (C_1), one D component (D_1), the ten E components, and the two E components (E_3 and E_4) (Fig. 11). In the tenth column of the first row (A10) in the second plate (P2) of the library L1 (hereinafter designated as location A10, P2, L1) there will be one C component (C_{10}), one D component (D_1), the ten B components, and the two E components (E_3 and E_4). In the tenth column of the eighth row (H10) in the second plate (P2) of the library L1 (hereinafter designated as location H10, P2, L1) there will be one C component (C_{10}), one D component (D8), the ten B components, and the two E components (E_3 and E_4).

In the first column of the first row (A1) in the third plate (P3) of the library L1 (hereinafter designated as location A1, P3, L1) there will be one C component (C_1), one D component (D_1), the ten B components, and the two E components (E_5 and E_6) (Fig. 12). In the tenth column of the first row (A10) in the third plate (P3) of the library L1 (hereinafter designated as location A10, P3, L1) there will be one C component (C_{10}), one D component (D_1), the ten B components, and the two E components (E_5 and E_6). In the tenth column of the eighth row (H10) in the third plate (P3) of the library L1 (hereinafter designated as location H10, P3, L1) there will be one C component (C_{10}), one D component (D_8), the ten B components, and the two E components (E_5 and E_6).

In the first column of the first row (A1 in the fourth plate (P4) of the library L1 (hereinafter designated as location A1, P4, L1) there will be one C component (C_1), one D component (D_1), the ten B components, and the two E components (E_7 and E_8) (Fig. 13). Likewise, in the tenth column of the first row (A10) in the fourth plate (P4) of the

library L1 (hereinafter designated as location A10, P4, L1) there will be one C component (C_{10}), one D component (D_1), the ten B components, and the two E components (E_7 and E_8). In the tenth column of the eighth row (H10) in the fourth plate (P4) of the library L1 (hereinafter designated as location H10, P4, L1) there will be one C component (C_{10}), one D component (D_8), the ten B components, and the two E components (E_7 and E_8).

A second complementary library is synthesised as follows. In the first column of the first row (A1) of the first plate (P1) of the library L2 (hereinafter designated as location A1, P1, L2), there will be ten C components, two D components (D_1 and D_2), one B component (B_1), and one E component (E_1) (Fig. 14). In the tenth column of the first row (A10) of the first plate (P1) of the library L2 (hereinafter designated as location A10, P1, L2), there will be the ten C components, two D components (D_1 and D_2), one B component (B_{10}), and one E component (E_1). In the first column of the eighth row (H1) of the first plate (P1) of the library L2 (hereinafter designated as location H1, P1, L2), there will be the ten C components, two D components (D_1 and D_2), one B component (B_1), and one E component (E_8). In the tenth column of the eighth row (H10) of the first plate (P1) of the library L2 (hereinafter designated as location H10, P1, L2) there will be the ten C components, two D components (D_1 and D_2), one B component (B_{10}), and one E component (E_8). Hence the matrix containing all ten columns and all eight rows are used to accommodate 1600 compounds in total.

In the first column of the first row (A1) of the second plate (P2) of the library L2 (hereinafter designated as location A1, P2, L2), there will be ten C components, two D components (D_3 and D_4), one B component (B_1), and one E component (E_1) (Fig. 15). In the tenth column of the first row (A10) of the second plate (P2) of the library L2 (hereinafter designated as location A10, P2, L2), there will be ten C components, two D components (D_3 and D_4), one B component (B_{10}), and one E component (E_1). In the first column of the second row (B1) of the second plate (P2) of the library L2 (hereinafter

designated as location B1, P2, L2), there will be ten C components, two D components (D_3 and D_4), one B component (B_1), and one E component (E_2). In the tenth column of the eighth row (H10) of the second plate (P2) of the library L2 (hereinafter designated as location H10, P2, L2), there will be ten C components, two D components (D_3 and D_4), one B component (B_{10}), and one E component (E_8).

In the first column of the first row (A1) of the third plate (P3) of the library L2 (hereinafter designated as location A1, P3, L2), there will be ten C components, two D components (D_5 and D_6), one B component (B_1) and one E component (E_1) (Fig. 16). In the tenth column of the first row (A10) of the third plate (P3) of the library L2 (hereinafter designated as location A10, P3, L2), there will be ten C components, two D components (D_5, and D_6), one B component (B_{10}), and one E component (E_1). In the first column of the second row (B1) of the third plate (P3) of the library L2 (hereinafter designated as location B1, P3, L2), there will be ten C components, two D components (D_5 and D_6), one B component (B_1), and one E component (E_2). In the tenth column of the eighth row (H10) of the third plate (P3) of the library L2 (hereinafter designated as location H10, P3, L2), there will be ten C components, two D components (D_5 and D_6), one B component (B_{10}), and one E component (E_8).

In the first column of the first row (A1) of the fourth plate (P4) of the library L2 (hereinafter designated as location A1, P4, L2), there will be ten C components, two D components (D_7 and D_8), one B component (B_1), and one E component (E_1) (Fig. 17). In the tenth column of the first row (A10) of the fourth plate (P4) of the library L2 (hereinafter designated as location A10, P4, L2), there will be ten C components, two D components (D_7 and D_8), one B component (B_{10}), and one E component (E_1). In the first column of the second row (B1) of the fourth plate (P4) of the library L2 (hereinafter designated as location B1, P4, L2), there will be ten C components, two D components (D_7 and D_8), one B component (B_1), and one E component (E_2). In the tenth column of the eighth row (H10) of the fourth plate (P4) of the library L2 (hereinafter designated as

location H10, P4, L2), there will be ten C components, two D components (D_7 and D_8), one B component (B_{10}), and one E component (E_8).

The strategy is thus based on the synthesis of two orthogonal sets of mixtures in solution. These solutions are each indexed in two dimensions. Thus the data from a scan identifies the most active compounds without the need for decoding or resynthesis.

The positional preferences of sub-units (in this case amino acids) are optimised with respect to all other variant positions simultaneously. The synergistic relationship between all four positions is realised and both positive, beneficial and negative, deactivating data are generated. This leads to families (sub-populations) cf. substrates and their sub-unit preferences. The data can be fed into molecular modelling programs to generate pharmacophoric descriptors that encompass both the desirable features (from the positive data) and indicate undesirable interactions (from the negative data sets).

Note that a one dimensional scan only indicates one position at a time as 'most active' and does not explore the synergistic relationship between positions.

The general methodology exemplified above with regard to the use of complementary combinatorial FRET libraries for the identification of proteolytic enzyme substrates, is equally applicable for identification of compounds from a library which interact with another active moiety.

Combinatorial libraries of compounds containing four variable groups B, C, D and E can be produced an interactions with active moieties detected using suitable reporters or markers.